

A PHYLOGENY OF *BEJARIA* (ERICACEAE: ERICOIDEAE)
BASED ON MOLECULAR DATA

Catherine M. Bush and Kathleen A. Kron

Wake Forest University
1834 Wake Forest Road
Winston-Salem, North Carolina 27106, U.S.A.
bushcm3@wfu.edu

ABSTRACT

Phylogenetic relationships of ten of the fifteen recognized species of *Bejaria* were examined using molecular (*ndhF* 5', *matK*; chloroplast and *waxy*; nuclear) data. Parsimony analyses were performed in PAUP for each gene, combined chloroplast data and total combined data. Bayesian analyses were performed in MrBayes for the *waxy*, combined chloroplast and total combined data sets. Bejarieae (*Bejaria*, *Bryanthus* and *Ledothamnus*) is not monophyletic in any analysis. The sampled members of *Bejaria* are monophyletic in all analyses with strong support. *Bejaria racemosa*, the only species from North America, is strongly supported as sister to all other *Bejaria* members.

RESUMEN

Las relaciones filogenéticas de 10 de las 15 especies reconocidas de *Bejaria* fueron examinadas utilizando datos moleculares (*ndhF* 5', *matK*, y *waxy*). Los análisis de parsimonia fueron realizados en PAUP para cada gen, para los datos de cloroplasto y para los datos combinados totales. Los análisis Bayesianos se realizaron en MrBayes para el gen nuclear *waxy*, los datos de cloroplasto y para los datos combinados totales. En nuestro análisis la tribu Bejarieae (*Bejaria*, *Bryanthus* y *Ledothamnus*) no es monofilética. En todos los análisis, los miembros muestreados de *Bejaria*, son monofiléticos con un soporte alto. *Bejaria racemosa*, la única especie de Norte América, fue resuelta como hermana de los otros miembros de *Bejaria*.

Bejaria Mutis ex. L. (Ericoideae: Ericaceae) contains fifteen species distributed in South America, Central America and the Caribbean with *B. racemosa* occurring in the southeast United States (Clemants 1995). The Ericaceae typically have a corolla that is fused basally and a five-merous corolla; therefore *Bejaria* was thought to be the most ancestral within the family due to its free petals and higher merosity (Stevens 2007; Camp 1941). This idea is not supported in recent studies (e.g., Kron et al. 2002) that show *Enkianthus* Lour. (Enkianthoideae) as sister to all other taxa in the Ericaceae. Among the eight recognized subfamilies in Ericaceae the Ericoideae clade contains five named groups (tribes): Bejarieae, Empetreae, Ericaceae, Phyllodoceae and Rhodoreae. Relationships among these five tribes are unresolved (Kron et al. 2002). *Bejaria*, *Bryanthus* S.G. Gmel. and *Ledothamnus* Meisn. comprise Bejarieae (Kron et al. 2002). *Ledothamnus* is a genus of nine species occurring in the Guyana Highlands and *Bryanthus* is monotypic, found only in Kamtchatka and Japan (Mabberley 1997).

The most recent taxonomic revision of *Bejaria* was based on morphological characters and there are two sections in the genus: sect. *Racemosae* and *Bejaria* (Clemants 1995). Fourteen of the species are in sect. *Bejaria*, while *B. racemosa* is the only member of sect. *Racemosae*, unique in its chartaceous leaves, the “stalked” appearance of its inflorescence (i.e., there is a relatively long area at the base of the raceme that does not contain leaves or flowers) and numerous distinct leaf anatomy characteristics (Clemants 1995).

After being discovered in 1767 in Colombia by Jose Celestino Mutis, *Bejaria* has been taxonomically revised three times in its entirety (Fedtschenko & Basilevskaja 1928; Mansfeld & Sleumer 1935; Clemants 1995). Fedtschenko and Basilevskaja (1928) focused their revision on the characteristics of pubescence and also inflorescence type. Mansfeld and Sleumer (1935) based their revision on plant indumentum and corolla size. Finally, Clemants (1995) based his monograph of the genus primarily on corolla shape, as opposed to the traditional use of indumentum and inflorescence characters.

Four species of *Bejaria*—*B. aestuans*, *B. resinosa*, *B. sprucei*, and *B. mathewsii*—are widespread in distribution, particularly *B. aestuans* which is found from Mexico to Bolivia (Clemants 1995). The other eleven

species are considered to be endemic and several are in need of conservation due to habitat destruction and spreading human habitation (Clemants 1995).

Bejaria is typically diagnosed by a combination of morphological features such as its seven-merous flowers, free petals, capsular fruits, non-appendaged anthers, and viscin threads among the pollen tetrads (Clemants 1995). Individually, many of these characteristics are shared with other members of Ericaceae (Stevens 1971). The only known potential morphological synapomorphy for the genus is tetracytic stomata (Stevens 1971).

An unusual characteristic of *Bejaria* is the presence of tubular corollas despite the fact that all species have separate petals (Clemants 1995). There are four recognized corolla shapes among the 15 species of *Bejaria* (Clemants 1995). The majority of species have campanulate and/or spreading corollas (*B. aestuans*, *B. cubensis*, *B. imthurnii*, *B. neblinensis*, *B. racemosa*, *B. steyermarkii*, *B. subsessilis*; Clemants 1995). Several other species exhibit tubular corollas (*B. ledifolia*, *B. mathewsii*, *B. resinosa*, *B. sprucei* and *B. zamorae*; Clemants 1995). Two species (*B. nana* and *B. tachirensis*) have small globose corollas (Clemants 1995). Finally, *B. infundibula* has a funnel-shaped corolla (Clemants 1995).

The variation seen in corolla shape within *Bejaria* may reflect different pollination modes and serve as reproductive isolating mechanisms for the separate species, but very little is actually known about the pollinators of the genus (Clemants 1995). For example, *B. resinosa* predominantly self-pollinates and only occasionally outcrosses (Kraemer 2001). When a biotic pollinator is involved, however, the long, tubular corollas of *B. resinosa* were thought to be pollinated by long-billed hummingbirds (Kraemer 2001). In a pollination study by Kraemer (2001), *B. resinosa* was visited more often by bumble bees (*Bombus funebris*). Bumble bees may pollinate *B. resinosa* more frequently than hummingbirds, but the birds may play an occasional, but significant role in long distance dispersal of pollen for the species (Kraemer 2001). In the study, short-billed hummingbirds were common but visited the flowers rarely, most likely due to the inaccessibility of nectar in the long tube (Kraemer 2001). Nectar robbers (flowerpiercers; *Diglossa humeralis*) were also a common visitor to these flowers but did not assist in pollination (Melampy 1987; Kraemer 2001). Out of the 15 species of hummingbirds at the site, only two were long-billed and both occurred infrequently in the area (Kraemer 2001). These data appear to suggest that hummingbird pollination is indeed rare for the long-tubed *B. resinosa* (Kraemer 2001).

Corollas that are spreading or campanulate are also visited by bees (Clemants 1995). For instance, *B. racemosa* (spreading or rotate corolla shape), from the southeastern United States, was visited by the common honey bee *Apis mellifera* (Clemants 1995). *Bejaria aestuans*, with a campanulate corolla, was visited by bees (Clemants 1995). No documentation for the successful pollination of these species by the visitors is available. Pollinators for the infundibular or globose corolla types are not known.

Ethnobotanically, species within the genus have been used on both continents independently as "fly paper" due to the thick resin secreted from glandular hairs on both the flowers and leaves (Clemants 1995). It has been shown that the resin on *B. racemosa* flowers is as strong as commercially produced fly adhesive (Eisner & Aneshansley 1983). Species of *Bejaria* have also been used for woodworking, medicinal purposes, and until late in the 19th century as a popular ornamental (Clemants 1995).

There has never been a published cladistic analysis of *Bejaria*. The goals of this study were to address: (1.) the monophyly of *Bejariaeae* and *Bejaria* using molecular data (2.) the relationships within the genus; particularly the relationship of *B. racemosa* to all other sampled species.

MATERIALS AND METHODS

Taxon sampling

Ten species (of the 15 recognized by Clemants 1995) of *Bejaria* were included in the molecular study. *Bejaria cubensis*, *B. nana*, *B. steyermarkii* and *B. tachirensis* were excluded because the chosen gene regions could not be successfully amplified from available material. *Bejaria neblinensis* is known only from the type locality (Cerro de la Neblina, Venezuela) and the very few specimens of this species were not available for loan or for DNA extraction.

For all analyses, 13 species representing other clades within Ericoideae were included (Table 1). In the chloroplast analyses (*ndhF*, *matK* and combined chloroplast analyses) ten species of *Bejaria* were included in the ingroup. For all analyses containing the nuclear *waxy* data set (*waxy* and total combined data analyses), *B. infundibula*, *B. mathewsii* and *B. sprucei* were excluded from the ingroup due to large amounts of missing data. Representative Ericoideae taxa were chosen based on previous work within the Ericaceae (Kron et al. 2002). All analyses were rooted with *Cassiope mertensiana*. *Cassiope* D. Don (Cassiopoideae) was found in previous analyses to be sister to the Ericoideae (Kron et al. 2002). Voucher information for the molecular study is presented in Table 1.

DNA extraction, amplification, and sequencing

Plant material originally dried for flavonoid analysis in 1983 was provided by Steven Clemants (Brooklyn Botanic Gardens). These leaves were air-dried and no other means of preservation was used (i.e., silica gel, refrigeration; S. Clemants, pers. comm.). DNA was extracted from these ~20 year old leaves and other material using an Epicentre Leaf Extraction kit (Epicentre Technologies, Madison, Wisconsin, U.S.A.) or by using the CTAB protocol of Doyle and Doyle (1987). Less degraded DNA was obtained from the "air"-dried leaves compared to similarly aged herbarium sheets. All extractions were cleaned with a Qiagen cleanup kit (Qiagen Sample and Assay Technologies, Valencia, California, U.S.A.).

Two chloroplast genes, *ndhF* and *matK*, were amplified using standard polymerase chain reaction (PCR) protocols and previously published primers (Olmstead et al. 1993; Steel & Vilgalys 1994). The *ndhF* gene encodes a subunit of the nicotinamide dehydrogenase complex and is ca. 2223 base pairs long (Olmstead & Sweere 1994; Alverson et al. 1999). The dehydrogenase complex functions in direct hydrogen transfer between the substrate and the nicotinamide ring (Cook & Cleland 1981). The *matK* gene encodes a ribosomal maturase and it is located in the intron of *trnK*, a gene that encodes the transfer RNA for lysine (Johnson & Soltis 1994). The *matK* gene is approximately 1500 base pairs long (Johnson & Soltis 1994). The nuclear gene included in this study is *waxy*, which encodes the granule-bound starch synthase (GBSSI; Miller et al. 1999). This gene has 13 translated exons and is approximately 4500 base pairs long (Peralta & Spooner 2001). The gene functions in the synthesis of amylose (Mason-Gamer et al. 1998). For this study, the region between exons 9 and 11 was amplified for a total of 628 base pairs (Miller et al. 1999; Peralta & Spooner 2001). *Bejaria aestuans*, *B. imthurnii*, *B. racemosa* and *B. resinosa* were cloned for the *waxy* gene and because no multiple copies were found the remainder of the species were not cloned. All PCR products were gel isolated and cleaned using Qiagen gel isolation kits (Qiagen Sample and Assay Technologies, Valencia, California, U.S.A.). Sequencing was performed on an ABI 3700 machine located at the DNA sequencing facility of Wake Forest University. All sequences were edited using Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI) and were aligned manually. GenBank accession numbers are listed in Table 1.

Phylogenetic analyses

Parsimony analyses were performed in PAUP* 4.0b2 (Swofford 1999). Characters were unordered and gaps within the molecular data were treated as missing data. All characters were equally-weighted and only those that were parsimony informative were included in the analyses. For *ndhF*, only the 5' end of the gene was used due to extensive missing data in several taxa in the 3' end. The total number of base pairs for the *ndhF* 5' gene region is 1318. The three gene regions (*ndhF*, *matK*, and *waxy*) were analyzed separately using the heuristic search option with 1000 replicates, TBR branch swapping, and random step-wise addition. Bootstrap analyses were used to estimate clade support and bootstrap scores (bt) were recorded (1000 replicates of TBR branch swapping with 100 replicates in the heuristic search; Felsenstein 1985). Conflict between trees was assessed by inspecting the results from different analyses for medium to high bootstrap scores (greater than 80%) that supported different relationships between the taxa. There was no strongly supported conflict between the two chloroplast genes and the *waxy* gene region so all data were combined for a total evidence analysis.

For the Bayesian analyses, each individual gene set was run in ModelTest 3.7 (Posada & Crandall 1998) to determine the best substitution type (Nst) and rate distribution model (rates) for each gene region.

TABLE 1. *Bejaria* and other species in the Tribe Bejariaceae sampled, sources of material, vouchers, and GenBank accession numbers for molecular analyses.

Taxon	Voucher	<i>ndhF</i>	<i>matK</i>	<i>waxy</i>
<i>Bejaria aestuans</i> Mutis ex. L.	Luteyn 14175 (NY)	DQ002362	DQ002346	DQ000589
<i>Bejaria imthurnii</i> N.E. Br.	Clemants & Delascio 2453b (NY)	DQ002363	DQ002347	DQ000590
<i>Bejaria infundibula</i> Clemants	Clemants & Fernandez 2007c (NY)	DQ002364	DQ002348	N/A
<i>Bejaria ledifolia</i> Bonpl.	Clemants 2483b (NY)	DQ002365	DQ002349	DQ000592
<i>Bejaria mathewsii</i> Fielding	Clemants 2046 (NY) & Gardner	DQ002366	DQ002350	N/A
<i>Bejaria racemosa</i> Vent.	Kron 2070 (NCU)	DQ002367	DQ002351	DQ000594
<i>Bejaria resinosa</i> Mutis ex. L.	Luteyn 14133 (NY)	DQ002368	DQ002352	DQ000595
<i>Bejaria sprucei</i> Meisn.	Clemants & Delascio 2479 (NY)	DQ002369	DQ002353	N/A
<i>Bejaria subsessilis</i> Benth.	Clemants 2278a (NY)	DQ002370	DQ002354	DQ000596
<i>Bejaria zamorae</i> Clemants	Bush 212 (LOJA)	DQ002371	DQ002355	DQ000597
<i>Bryanthus gmelinii</i> D. Don	Stevens DNA 192 (WFU)	EU669881	AF440413	EU669884
<i>Cassiope mertensiana</i> (Bong.) G. Don	s.n. (AA 75-83)	DQ002372	U61346	DQ000598
<i>Ceratiola ericoides</i> (Michx.)	Kron 2069 (WFU)	DQ002373	U61334	DQ000599
<i>Elliottia bracteata</i> (Maxim.) Hook. f.	s.n. (RBGK 1979-5019)	DQ002374	U61339	DQ000600
<i>Empetrum atropurpureum</i>	s.n. (RBGK 1988-433) Fernald & Wiegand	DQ002375	U61355	DQ000601
<i>Kalmia angustifolia</i> L.	Kron DNA156 (WFU)	DQ002376	DQ002356	DQ000602
<i>Kalmiopsis leachiana</i>	Kron DNA177 (WFU) (Henderson) Rehder	DQ002377	U61323	DQ000603
<i>Ledothamnus guyanensis</i> Meisn.	Picon & Williams 2910 (NY)	EU669882	AF440419	EU669885
<i>Phyllodoce caerulea</i> Bab.	s.n. (RBGK 1940-1013)	DQ002378	DQ002357	DQ000604
<i>Phyllodoce empetriformis</i> D. Don	s.n. (RBGK 1978-2120)	DQ002379	DQ002358	DQ000605
<i>Rhododendron grande</i> Wight	s.n. (RBGK 1969-8606)	DQ002383	DQ002360	EU669886
<i>Rhododendron hippophaeoides</i>	s.n. (RBGK 1932-1022) Balf.f. & W.W. Sm.	EU669883	U61353	EU669887
<i>Rhodothamnus chamaedistus</i> Rchb.	s.n. (RBGK 1989-459)	DQ002381	U61321	DQ000607

Three separate analyses were performed in MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003): *waxy*, combined chloroplast and total combined data. The individual *ndhF* and *matK* analyses were not performed using Bayesian because the results from each were predominantly unresolved in the parsimony analysis and the combined chloroplast tree represents both data sets. For the multiple gene analyses, the genes were partitioned in MrBayes and the appropriate models were applied for each gene region. Each analysis ran for 1,000,000 generations and was sampled every 100 generations. The burn-in period was the first 2,500 sampled generations. The model used for each gene region is as follows: *ndhF*: HKY+G; *matK*: TVM+G and *waxy*: HKY+G. Posterior probability values (ρ P) were calculated in MrBayes.

RESULTS

Analysis of chloroplast sequences

The aligned *ndhF* data set for 23 taxa was 1318 base pairs long. For this parsimony analysis, 1719 most parsimonious trees were found (L = 531; C.I. = 0.59; R.I. = 0.72; data not shown). There were 217 informative characters (16.5 %). The aligned *matK* data was comprised of 1541 characters and included the same taxa as in the *ndhF* analysis. In the *matK* analysis, 198 most parsimonious trees were recovered (L = 470; C.I. = 0.63; R.I. = 0.78; data not shown) and 216 characters were informative (14.0 %). Results of the separate *ndhF* and *matK* parsimony analyses were similar in support and topology to the combined chloroplast analysis so only the results of the latter will be discussed.

The combined chloroplast parsimony analysis resulted in 431 informative characters (2859 included

base pairs) and 9 most parsimonious trees ($L = 1012$; C.I. = 0.60; R.I. = 0.74). *Bejaria* is found to be monophyletic in this analysis (100% bt; Fig. 1). *Bejaria infundibula* is sister to *B. mathewsii* (91% bt) and this clade is in a polytomy with *B. imthurnii* and *B. subsessilis* (73% bt; Fig. 1). The four taxa in this clade are placed in a trichotomy with *Bejaria aestuans* and *B. zamorae* (62% bt; Fig. 1). Sister to this clade is *B. resinosa* (100% bt), followed by *B. sprucei* (65% bt) and *B. ledifolia* (50% bt; Fig. 1). Finally, *B. racemosa* is sister to all other *Bejaria* members sampled (Fig. 1). Outgroup relationships based on this analysis show Clade A (Phyllodoceae, Kron et al. 2002) as sister to *Bejaria*, but without support (Fig. 1). Clade B (Empetreae + Rhodoreae, Kron et al. 2002) is placed sister to Clade A + *Bejaria* and Clade C is sister to the remaining sampled taxa (Fig. 1).

A monophyletic *Bejaria* was also recovered in the Bayesian combined chloroplast analysis (100% $_pP$; Fig. 2). In this phylogeny, *B. imthurnii* and *B. subsessilis* are sister to each other (52% $_pP$) as are *B. infundibula* and *B. mathewsii* (100% $_pP$) and the two clades are sister to each other (97% $_pP$; Fig. 2). *Bejaria aestuans* and *B. zamorae* are in a polytomy with the previous clade (88% $_pP$; Fig. 2). Sister to this larger clade is *B. resinosa* (100% $_pP$; Fig. 2). *Bejaria ledifolia* and *B. sprucei* are unresolved with respect to this clade (100% $_pP$; Fig. 2). Finally, *B. racemosa* is sister to all other *Bejaria* sampled (100% $_pP$; Fig. 2). The relationship of Clade A to *Bejaria* is the same in this analysis as in the parsimony results (Figs. 1, 2). However in this analysis Clade B is sister to Clade C, although with low posterior probability (Fig. 2).

Analysis of waxy (nuclear) sequences

The aligned portion of *waxy* for 20 included taxa was 628 bases long (region between exons 9–11). The parsimony analysis resulted in 450 trees with a length of 175 (C.I. = 0.71; R.I. = 0.83). There were 93 informative characters in this analysis (14.8%). This analysis also supported a monophyletic *Bejaria* (100% bt, Fig. 3). It places *B. aestuans*, *B. imthurnii*, *B. ledifolia*, *B. resinosa*, *B. subsessilis*, and *B. zamorae* in a polytomy (94% bt) with *B. racemosa* sister to this clade (100% bt; Fig. 3).

In the Bayesian analysis of the *waxy* data, *Bejaria* is monophyletic (100% $_pP$; Fig. 4) but there is very little resolution within the genus. *Bejaria racemosa* is sister to all other *Bejaria* members, which are in a polytomy (99% $_pP$; Fig. 4). *Ledothamnus guyanensis* is sister to *Bejaria* (89% $_pP$; Fig. 4). Although placement of the outgroup taxa varies between parsimony and Bayesian analyses neither of these results are strongly supported.

Analysis of combined nuclear and chloroplast sequences:

The combined data set was 3487 aligned bases long. There were 513 informative characters (14.7%). The parsimony analysis produced 6 trees 1142 steps long (C.I. = 0.62; R.I. = 0.73). In this combined analysis, *Bejaria* is monophyletic with 100% bootstrap support (Fig. 5). *Bejaria imthurnii* and *B. subsessilis* are sister clades (95% bt) inside a polytomy including *B. aestuans* and *B. zamorae* (89% bt; Fig. 5). Sister to this polytomy is *B. resinosa* (100% bt; Fig. 5) and sister to this larger group is *B. ledifolia* (85% bt; Fig. 5). Finally, *B. racemosa* is sister to the remaining taxa sampled (Fig. 5). Among the outgroup taxa Clades B and C are placed as sister with very low support (56% bt) and sister to this clade is Clade A (Fig. 5).

In the Bayesian analysis of all combined data, *Bejaria* is monophyletic (100% $_pP$; Fig. 6). The clade *B. imthurnii* + *B. subsessilis* (100% $_pP$) is in a polytomy with *B. aestuans* and *B. zamorae* (100% $_pP$; Fig. 6). Sister to this clade is *B. resinosa* (100% $_pP$) followed by *B. ledifolia* (100% $_pP$; Fig. 6). Finally, *B. racemosa* is sister to all other *Bejaria* members sampled (Fig. 6). Similar to the parsimony analysis Clades B and C are placed as more closely related to each other than to Clade A (Figs. 5, 6). However, the Bayesian analysis places Clade A sister to *Bejaria* (Fig. 6).

DISCUSSION

Both parsimony and Bayesian total evidence analyses show *Bejaria* as monophyletic and *B. racemosa* as sister to the remaining *Bejaria* sampled. Deeper nodes of the tree prevent conclusions about the closest relatives to *Bejaria* as can be seen by the difference in placement of Clade A as sister to *Bejaria* in the Bayesian results (Fig. 6) or as (Clade A (Clade B + Clade C)) in the parsimony results (Fig. 5). Clade C contains two members of the Bejariaeae as recognized by Kron et al., (2002). In their study they circumscribe Bejariaeae

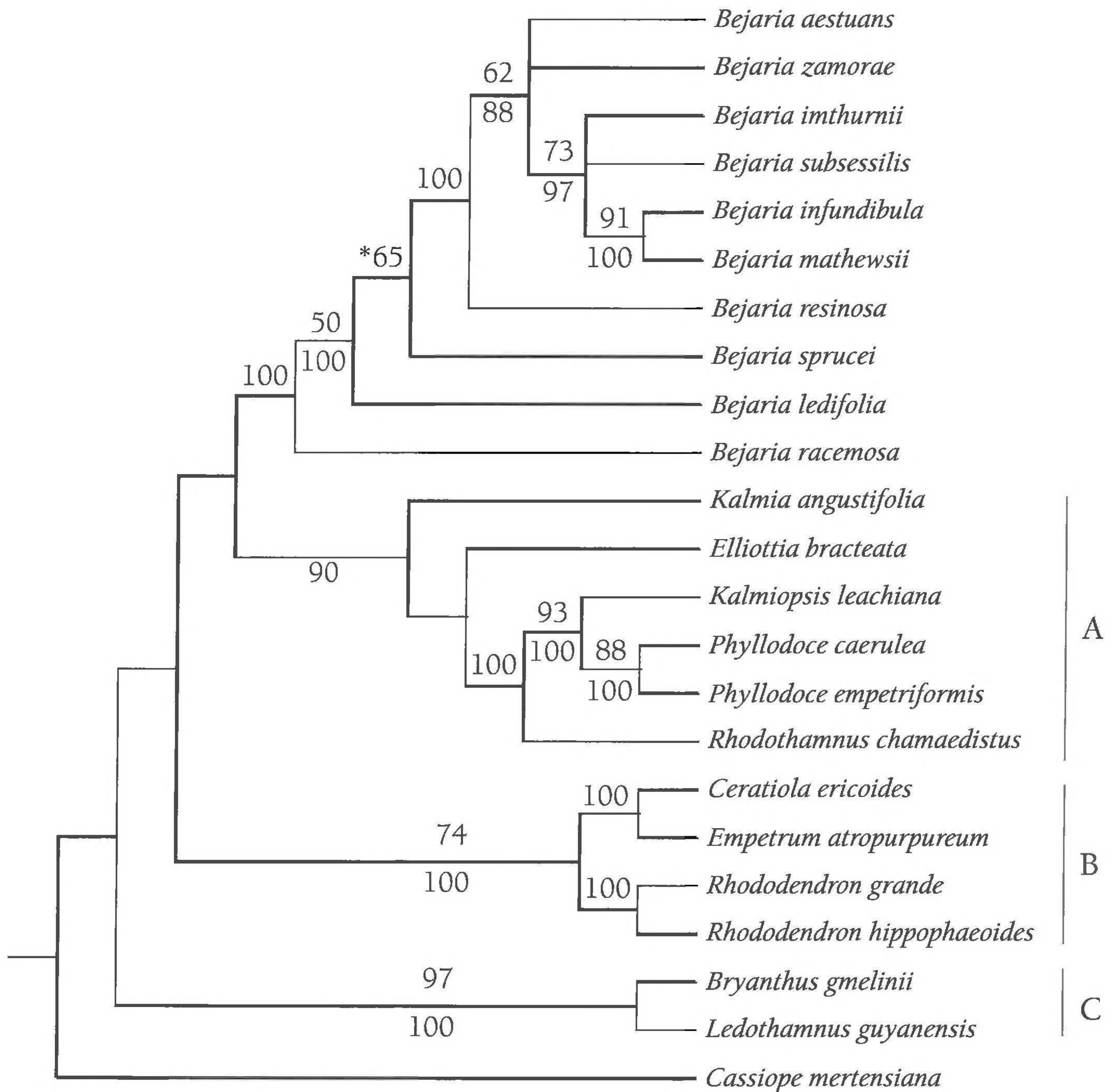


FIG. 1. Strict consensus of 9 most parsimonious trees ($L = 1012$, $CI = 0.60$, $RI = 0.74$) obtained in the analysis of the combined chloroplast genes ($5'$ *ndhF* and *matK*). When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. An asterisk next to a value indicates that the node is not present in the other analysis (parsimony or Bayesian; see text). A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

as containing *Bejaria*, *Bryanthus* and *Ledothamnus* and map a single (homoplasious) synapomorphy for the tribe, that of separate petals. This character also occurs in other genera within the Ericaceae (i.e., *Elliottia*, *Ceratiola*, *Corema*, *Chimaphila*, *Orthilia* and *Pyrola*; Kron et al. 2002) some of which are represented as outgroups in this study.

Bejariaeae is not monophyletic in any analysis in this study although the relationships outside of the terminal groups are poorly supported. Of the Bejariaeae, only *Ledothamnus* and *Bryanthus* are strongly supported as closely related in both the parsimony and Bayesian results of the total evidence and chloroplast analyses (Figs. 1, 2, 5, 6). This clade has several potential morphological synapomorphies such as ericoid leaves, adaxial calyx stomata, a style articulated with the ovary and the lack of endothecium (Kron et al.

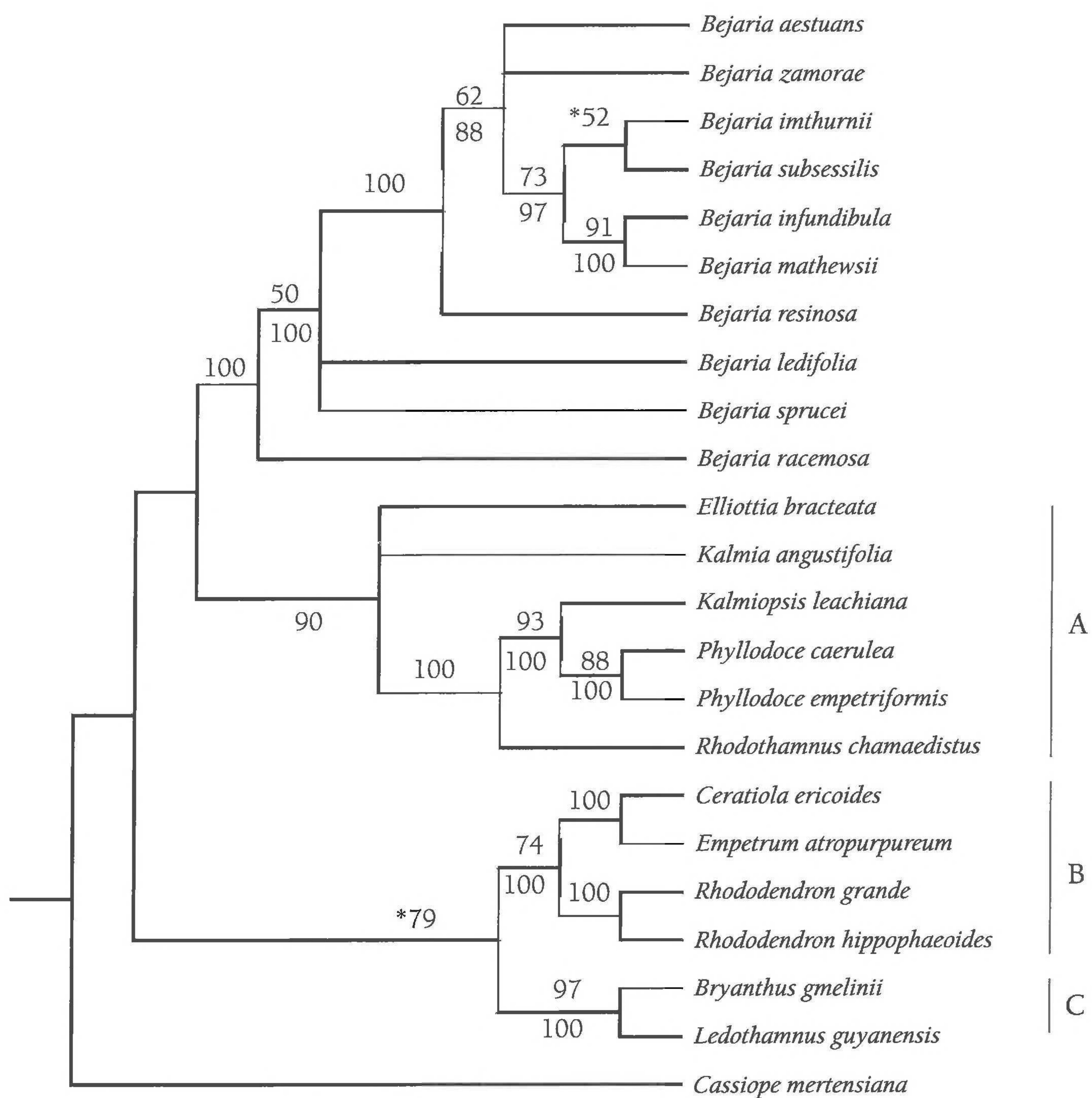


FIG. 2. Single consensus tree from a partitioned Bayesian analysis of the 5' *ndhF* and *matK* data sets. When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. An asterisk next to a value indicates that the node is not present in the other analysis (parsimony or Bayesian; see text). A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

2002). Parsimony and Bayesian analyses of *waxy* data do show *Ledoethamnus* and *Bryanthus* in different positions within the trees (Figs. 3, 4), but these are not well supported either by bootstrap or posterior probability measures.

In his monograph of *Bejaria*, Clemants (1984) performed a morphological cladistic analysis of all 15 recognized species of *Bejaria* using 16 characters, eight of which were related to floral morphology. Variation within Rhododendroideae (Kron 1997) was so significant that its use as an outgroup required designating *B. racemosa* as a functional outgroup for characters 5–16 in the study (Clemants 1984). Only four characters did not vary in the subfamily outgroup (Rhododendroideae; Clemants 1984). The cladogram produced from

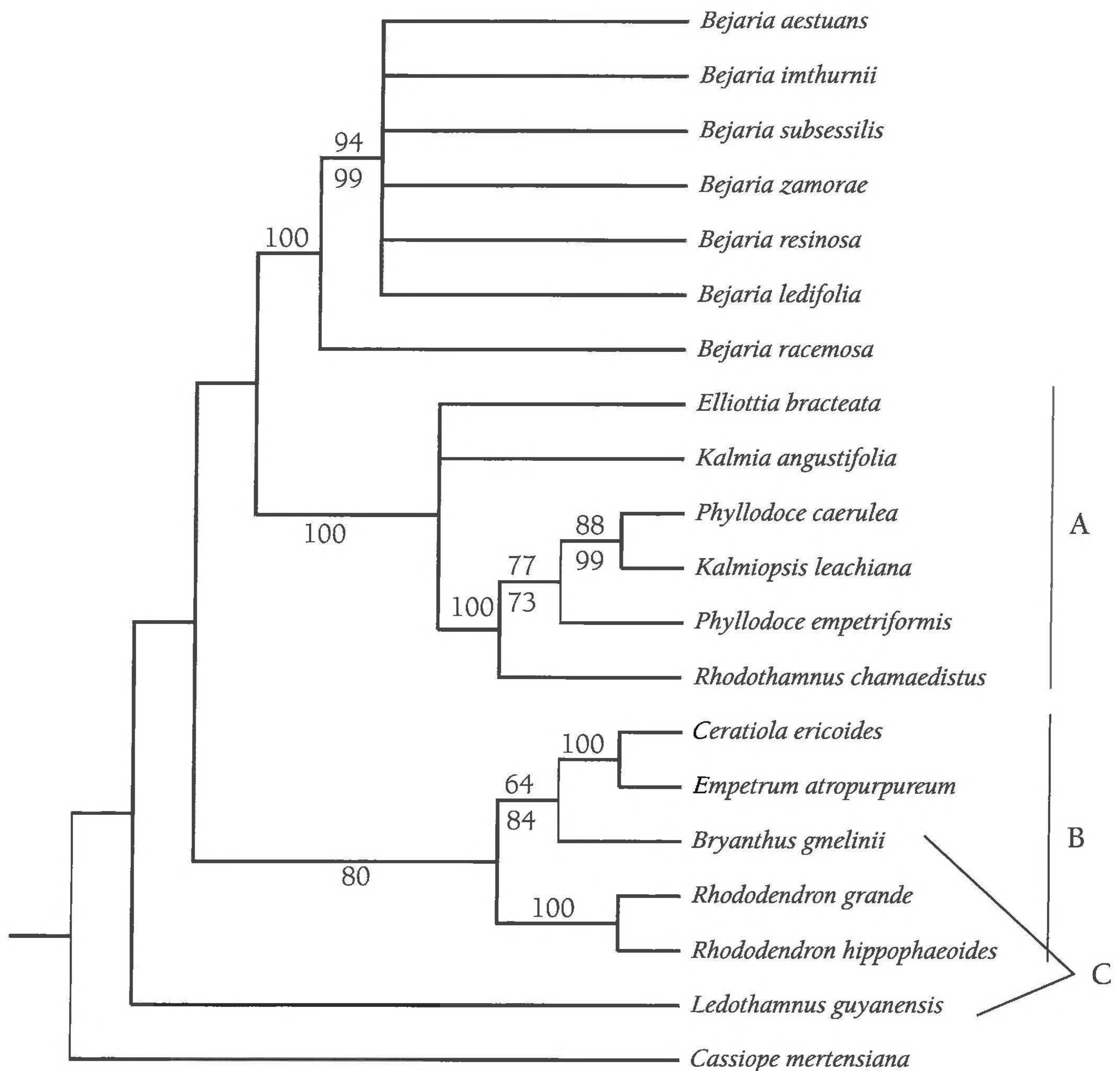


FIG. 3. Strict consensus of 1369 most parsimonious trees ($L = 193$, $CI = 0.74$, $RI = 0.85$) obtained in the analysis of the *waxy* data. *Bejaria infundibula*, *B. mathewsii* and *B. sprucei* were excluded from this analysis due to large amounts of missing data in the *waxy* gene. When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

the four characters placed *B. racemosa* as sister to all other *Bejaria* species (Clemants 1984). In the cladogram based on these four characters, a mucilaginous epidermis was a synapomorphy for all species in Central, South America and Cuba (Clemants 1984). *Bejaria racemosa* had the autapomorphy of stomata on both leaf surfaces (Clemants 1984). Flower shape was divided into three states: tube-shaped, cup-shaped and funnel-shaped (Clemants 1984). *Bejaria infundibula* alone possessed a funnel-shaped corolla, *B. nana* and *B. tachirensis* possessed cup-shaped corollas and the presence of tube-shaped corollas was a synapomorphy for the clade containing *B. ledifolia*, *B. mathewsii*, *B. resinosa*, *B. sprucei*, and *B. zamorae* (Clemants 1984). *Bejaria racemosa* and *B. nana* share the presence of an apical bracteole (compared to basal or medial) but this character has possibly evolved twice within *Bejaria* (Clemants 1984). The analyses in the present study represent a much smaller sample of *Bejaria* than Clemants (1984) due to problems with DNA extractions and PCR amplifica-

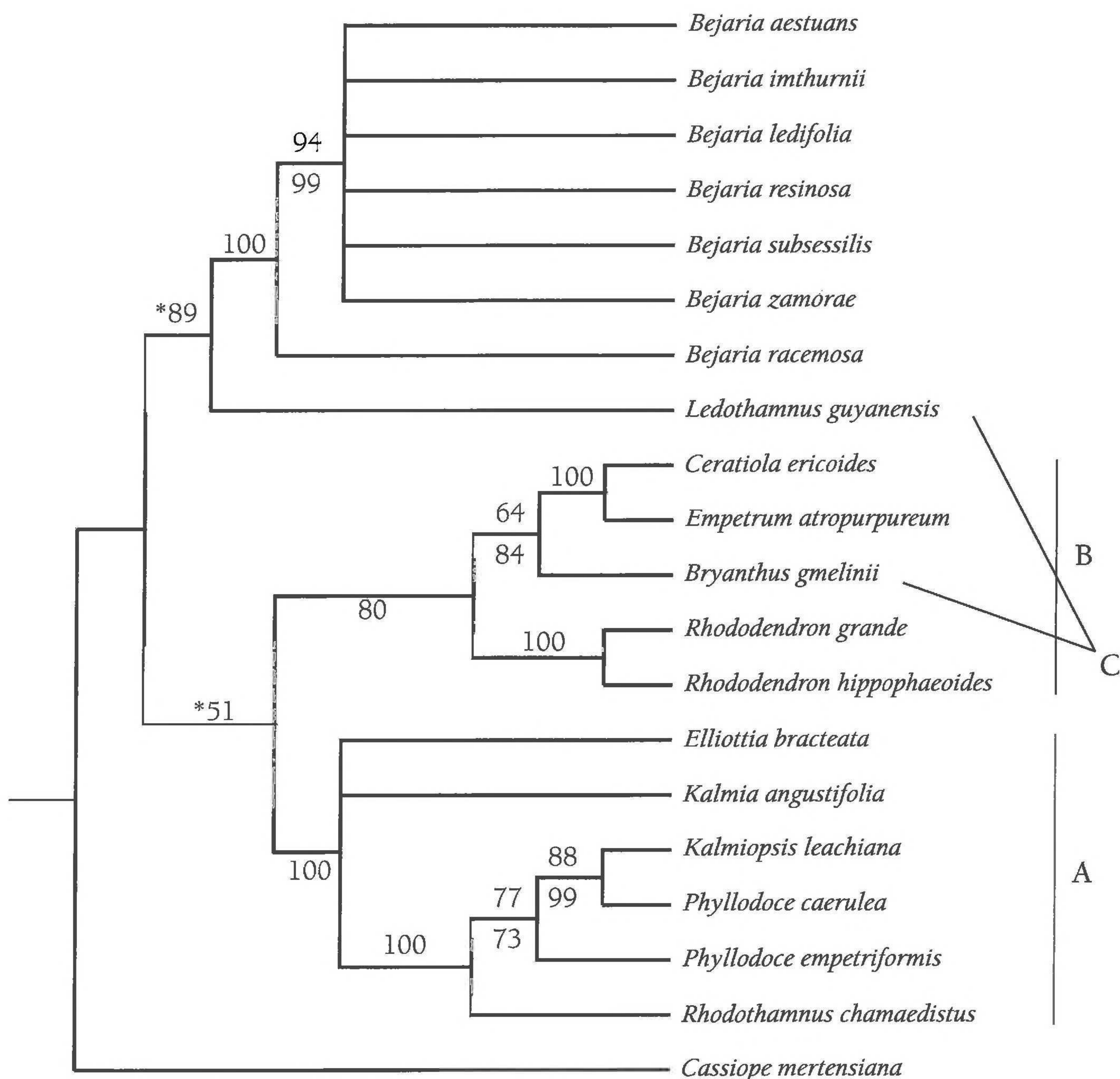


FIG. 4. Single consensus tree from a Bayesian analysis of the *waxy* data set. *Bejaria infundibula*, *B. mathewsii* and *B. sprucei* were excluded from this analysis due to large amounts of missing data in the *waxy* gene. When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. An asterisk next to a value indicates that the node is not present in the other analysis (parsimony or Bayesian; see text). A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

tion of old material as well as obtaining rare taxa. However our outgroup taxa include many members of previously circumscribed Rhodendroideae (with the exception of Empetreae) so our results are useful when compared to those of Clemants' (1984).

The revision of *Bejaria* by Clemants (1995) differed from previous revisions of the genus due to its focus on corolla shape as a delimiting character for species recognition. However, the total evidence analyses in this study (Figs. 5, 6) indicate that a tubular corolla such as that found in *B. ledifolia*, *B. resinosa*, and *B. zamorae* has likely evolved as least twice within *Bejaria*. The placement of clades with taxa that possess bell-shaped or spreading corollas (*B. aestuans*, *B. imthurnii*, and *B. subsessilis*) intercalated within clades that represent

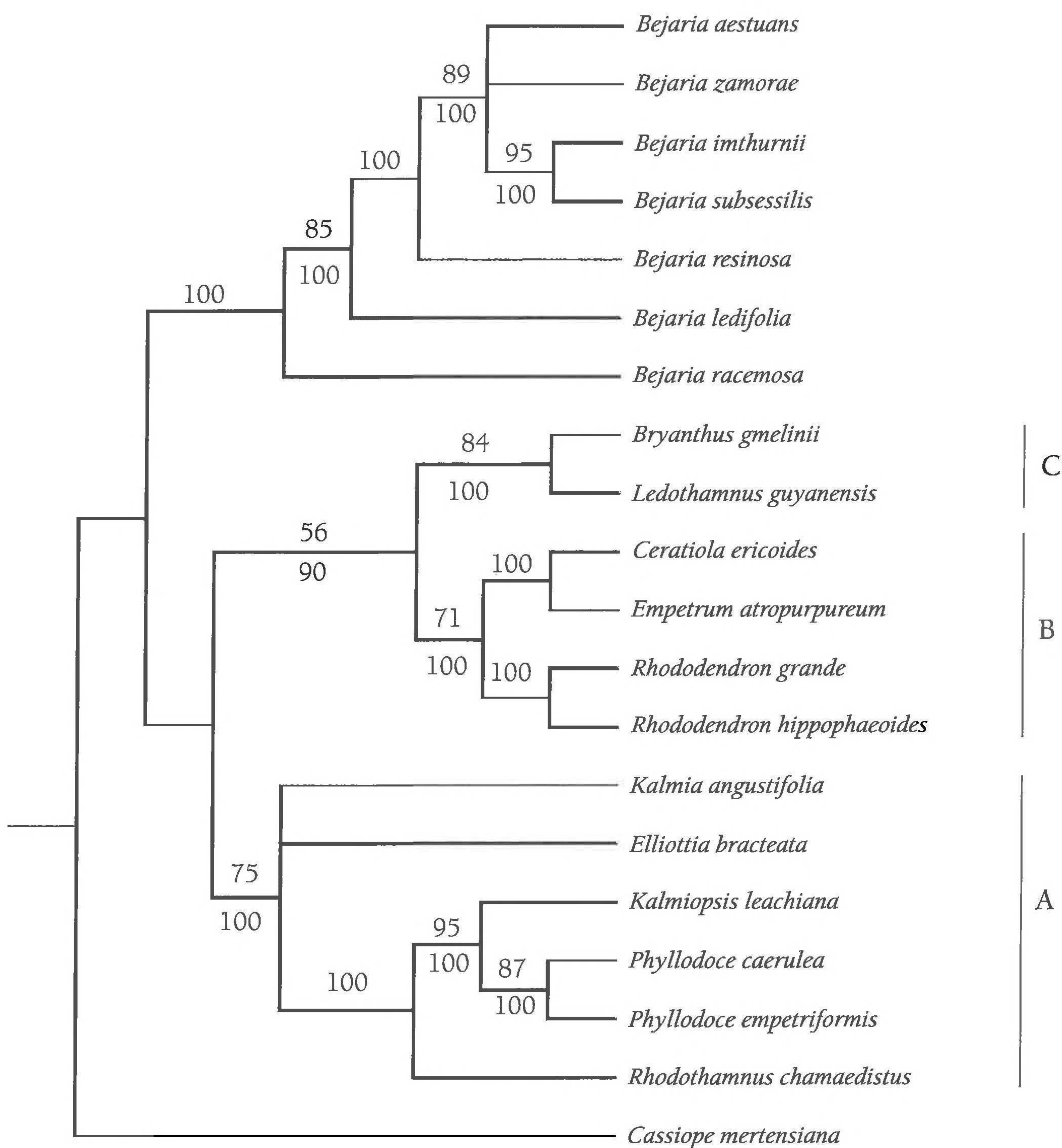


FIG. 5. Strict consensus of 6 most parsimonious trees ($L = 1142$, $CI = 0.62$, $RI = 0.73$) obtained in the analysis of the *ndhF* 5', *matK* and *waxy* data combined. *Bejaria infundibula*, *B. mathewsii* and *B. sprucei* were excluded from this analysis due to large amounts of missing data in the *waxy* gene. When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

taxa with tubular corollas indicates that corolla shape designations may not be phylogenetically informative within the genus.

Phylogenetic relationships among the species of *Bejaria* sampled in this study are identical in both the Bayesian and parsimony total data analyses and are well supported. In our analyses a novel, strongly supported relationship between *B. imthurnii* and *B. subsessilis* was found (Figs. 5, 6), although Clemants' (1984) study does not support this relationship. Instead, his results indicate that *B. subsessilis* is sister to *B. cubensis*

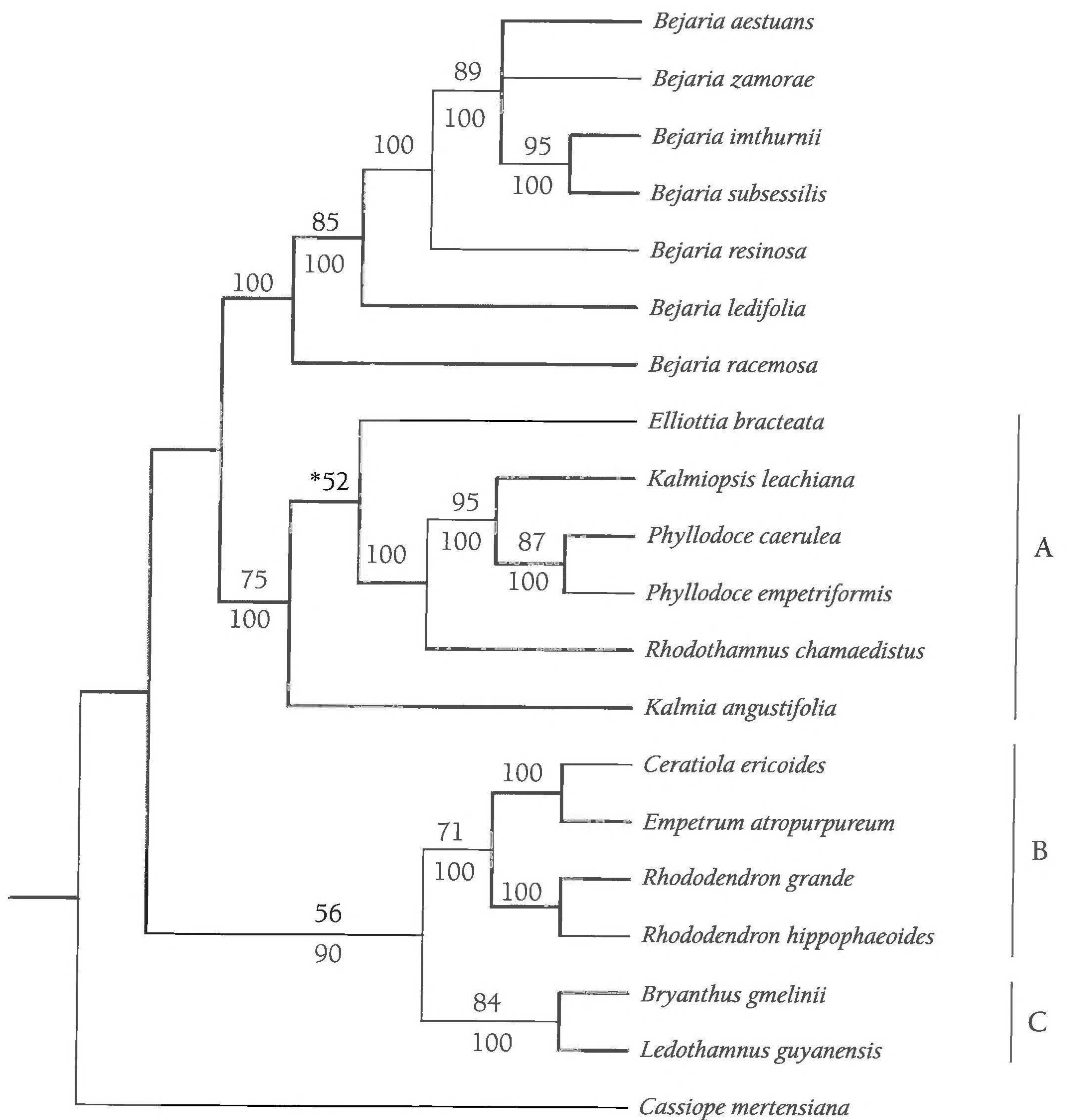


FIG. 6. Single consensus tree from a partitioned Bayesian analysis of the *ndhF5'*, *matK* and *waxy* data sets. *Bejaria infundibula*, *B. mathewsii* and *B. sprucei* were excluded from this analysis due to large amounts of missing data in the *waxy* gene. When two numbers on a branch are present, numbers above the branches represent bootstrap values greater than 50% and numbers below the branches indicate posterior probabilities. An asterisk next to a value indicates that the node is not present in the other analysis (parsimony or Bayesian; see text). A single number on top of the branch without an asterisk indicates that the support and topology are the same in both parsimony and Bayesian analyses. Finally, a single number below the branch without an asterisk indicates that the topology at that node is present in both analyses (parsimony and Bayesian) but the node is only supported in the Bayesian analysis (posterior probability shown). Clades A, B and C are labeled (see text).

due to the shared presence of a long floral bract (data not shown). *Bejaria imthurnii* and *B. subsessilis* do not share geographic ranges: *B. imthurnii* is endemic to the summits of Cerro Roraima and Cerro Kukenán on the border of Venezuela, Brazil and Guyana while *B. subsessilis* is endemic to Loja, Ecuador (Clemants 1995). *Bejaria imthurnii* shares some diagnostic morphological characters with *B. steyermarkii* and *B. neblinensis* (Clemants 1995). The three taxa share small, short-petioled leaves and campanulate corollas (Clemants 1995). Clemants (1995) noted that *B. subsessilis* possessed some characters that were intermediate between

B. aestuans and *B. resinosa* (e.g., rachis similar to *B. aestuans*, subsessile leaves with obtuse leaf bases like *B. resinosa*) suggesting that *B. subsessilis* possibly arose as a hybrid between *B. aestuans* and *B. resinosa*. In addition, some populations of *B. subsessilis* have distorted tubular corollas and two of the four pollen grains in the tetrads are aborted (Clemants 1995).

The relationships of *Bejaria aestuans* and *B. zamorae* to the *B. imthurnii* + *B. subsessilis* clade (89% bt, 100% ρ P respectively; Figs. 5, 6) are not resolved. While these species share several morphological traits, whether or not they indicate relationship remains to be tested. *Bejaria aestuans* is the most geographically widespread and morphologically variable species in the genus: it is found from Mexico to Bolivia (Clemants 1995). *Bejaria zamorae*, conversely, is endemic to the Rio Zamora valley in Ecuador (Clemants 1995). *Bejaria zamorae* shares several taxonomically diagnostic characters with both *B. aestuans* and *B. sprucei* (not sampled in the total data analyses; Clemants 1995). *Bejaria zamorae* has distinct thin and long pedicels and a strongly tubular corolla like *B. sprucei*. However, *B. zamorae* also exhibits slightly exerted stamens and a 7-merous corolla similar to *B. aestuans* (Clemants 1995). *Bejaria sprucei* exhibits a disjunct geographic distribution both north and south of the Ecuadorian volcanic region: one region includes the Guyana Highlands area and the mountains near Táchira and Anzoátegui, Venezuela and the other geographic region for the species is in northeastern Peru near San Martín (Clemants 1995).

Bejaria resinosa is sister to the clade containing *B. aestuans*, *B. zamorae*, *B. imthurnii* and *B. subsessilis* (Figs. 5, 6). *Bejaria resinosa* shares tubular flowers and long calyx lobes with *B. ledifolia* and *B. mathewsii* (not sampled in total evidence analyses) and these are considered indicators of relationship by Clemants (1995). In general, our results agree that *B. ledifolia* and *B. resinosa* are generally related (Figs. 5, 6) but they are not placed as sister in our analyses, indicating that such characters as obtuse to truncate leaf bases, short petioles, and long calyx lobes may not be phylogenetically informative.

This study indicates that additional approaches to resolve these closely related species are desired. A more complete sampling of the species within the genus, especially the rare and endemic taxa is important. Additional morphological cladistic work that builds on Clemants' (1984, 1995) studies are likely to improve our understanding of relationships within the genus and hyper-variable chloroplast or nuclear markers may be able to differentiate among these taxa more clearly. Population-level studies of widespread taxa such as *B. aestuans* and *B. mathewsii* which include samples from the geographic and morphological extremes of each species could clarify the potentially complicated relationships within this genus.

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